

Preparation of chromosomal DNA

Materials, for two strains:

10 mL TNE buffer
2 mL TNEX
200 μ L Lysozyme (5 mg/ml)
50 μ L Proteinase K (20 mg/ml)
200 μ L 5M NaCl
96% EtOH
100mL dH₂O

Don't vortex
chromosomal DNA
(after step 6) or it
will shear / break.

1. Harvest 500 μ L culture: Spin down cells (5 min at 7000 g) and decant.
2. Wash with 1 ml TNE: Resuspend in 1 ml TNE; vortex; spin down cells (5 min at 7000 g); decant.
3. Resuspend pellet in 270 μ L TNEX.
4. Add 30 μ L of a freshly prepared lysozyme solution (5 mg/ml in H₂O).
5. Add 7.5 μ L Proteinase K solution (20 mg/ml in H₂O)
6. Incubate for 90 min at 37°C and then at 65°C for another 90 min.
7. Add 15 μ L 5 M NaCl and mix gently by inverting the tube.
8. Add gently 1 ml 96% EtOH. After 2-3 min mix gently and incubate 15 min at -20°C.
9. Spin at 15000 g for 10 min at 4°C.
10. Wash pellet with 1 ml ice cold 96% EtOH. Be careful! Don't loose the DNA!
11. Spin at 15000 g for 10 min at 4°C.
12. Remove remaining EtOH by pipetting followed by incubation at 37°C for 3-5 min with the lid open (do not over dry pellet).
13. Add 500 μ L dH₂O and mix gently by inverting the tube. Store at 20°C o.n. to dissolve the DNA; then at -20°C

16 S rDNA PCR amplification on chromosomal DNA from 2 isolates.

Materials

Sterile MilliQ water

10xPCR buffer

dNTP 10mM

9F primer, 5'-GAGTTTGATCCTGGCTCAG-3' (100µM)

1512R primer, 5'-ACGGCTACCTTGTTACGACTT-3' (100µM)

DMSO

Taq-polymerase

Important – Keep everything on ice until the PCR is running and work sterile!

The 16S rDNA from 2 isolates is amplified by the use of PCR with the primers

9f and **1512r**

1. Prepare a PCR master mix with 4 x each of the components written below, except the DNA template and Taq-polymerase.
2. Pipette 48,5 µl of the master mix in 3 PCR tubes and add 1 µl chromosomal DNA solution to 2 of the tubes; one tube for each species! The third tube is a negative control without DNA.
3. The Taq-polymerase should be added just before the run. The master mix is made in excess to cover losses during pipetting.

The final content of each of the 3 samples should be:

34.5 µl sterile MilliQ water

5 µl PCR buffer (*tag* buffer with (NH₄)₂SO₄, no MgCl₂)

2 µl dNTP

1 µl 9F primer

1 µl 1512R primer

1 µl DMSO

4 µl MgCl₂

1 µl DNA template (or dH₂O in the case of the negative control!)

0.5 µl Taq-polymerase

The PCR is done with the following temperature profile: 95°C 5 min; 30 cycles of:

94°C 30 sec,

53°C 30 sec,

72°C 90 sec,

72 °C 5 min;

thereafter 4°C until storage at -20°C.

PCR products are sent for sequencing